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Role of glucocorticoids in the maturation of the rat renal Na^+/H^+ antiporter (NHE3)

NEENA GUPTA, SAHAR R. TARIF, MOUIN SEIKALY, and MICHEL BAUM

Department of Pediatrics and Internal Medicine, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA

Role of glucocorticoids in the maturation of the rat renal Na^+/H^+ antiporter (NHE3).

Background. Neonates have a lower Na^+/H^+ antiporter activity on the apical membrane of proximal tubule than that of adults. The maturational increase in Na^+/H^+ antiporter activity occurs at the time when there is a rise in serum glucocorticoid levels in rats. The purpose of the present study was to examine whether glucocorticoids are responsible for the postnatal increase in Na^+/H^+ antiporter activity.

Methods. Nine-day-old Sprague-Dawley rats were compared with rats studied at 30 days of age who had either a sham operation or adrenalectomy (ADX) at nine days of age and with rats that had an adrenalectomy and physiologic corticosterone replacement (ADX-Cort) to determine whether glucocorticoid deficiency prevented the maturational increase in Na^+/H^+ antiporter activity. Na^+/H^+ antiporter activity was measured in proximal convoluted tubules perfused in vitro by the change in cell pH (pH_i) following luminal sodium removal. NHE3 mRNA abundance was measured using Northern blot analysis, and NHE3 protein abundance was measured by immunoblot.

Results. Na^+/H^+ antiporter activity was 93.8 ± 17.7, 157.0 ± 18.0, 356.7 ± 29.9, and 402.5 ± 14.5 pmol/mm · min in nine-day-old, ADX, ADX-Cort, and sham control groups, respectively. The ADX-Cort and sham control were higher than the 9-day-old and the 30-day-old ADX group ($P < 0.05$). Brush-border membrane NHE3 protein abundance in the nine-day-old and ADX groups were sixfold less than ADX-Cort and sham control groups ($P < 0.001$). Nine-day-old neonates had fivefold less renal cortical NHE3 mRNA than the ADX, ADX-Cort, and sham-operated control groups ($P < 0.01$).

Conclusions. These data demonstrate that glucocorticoids play a role in the postnatal maturation of the proximal tubule Na^+/H^+ antiporter activity and brush-border membrane NHE3 protein abundance. Glucocorticoid deficiency does not completely prevent the maturational increase in Na^+/H^+ antiporter activity and does not affect NHE3 mRNA abundance.

The proximal tubule is responsible for reclaiming 80% of the filtered bicarbonate. This is predominantly mediated by the Na^+/H^+ exchanger and to a lesser extent by an H^+-ATPase on the apical membrane of the proximal tubule [1, 2]. The rate of bicarbonate reabsorption and Na^+/H^+ exchanger activity in neonatal proximal tubules is lower than that in adults [3–6]. In addition, the Na^+/H^+ antiporter, in conjunction with a Cl^-/base exchanger, mediates transcellular, active NaCl transport in this nephron segment [7, 8]. Studies have also suggested that the postnatal maturation of the basolateral Na^+, K^+-ATPase activity is secondary to the developmental increase in the proximal tubule Na^+/H^+ exchanger [9].

To date, six isoforms of the Na^+/H^+ exchanger (NHE) have been cloned [10–19]. NHE1 is present on the basolateral membrane [20]. NHE3 is present on the apical membrane of the proximal tubule and is responsible for most of the luminal proton secretion in this nephron segment [21–25]. There also may be other amiloride-sensitive, sodium-dependent proton secretory mechanisms on the apical membrane of the proximal tubule that have not been characterized [25]. It has been shown that there is an increase in NHE3 but not NHE-1 mRNA and protein abundance with postnatal maturation [26].

The factors that increase proximal tubule Na^+/H^+ antiporter activity and NHE3 abundance during postnatal maturation are unknown. Glucocorticoid levels are lower in the neonate than in the adult [27]. There is a concordant rise in serum glucocorticoid levels and Na^+/H^+ antiporter activity and NHE3 protein and mRNA abundance during postnatal development in the rat [6, 27]. The purpose of this study was to examine whether glucocorticoids play a role in the maturation of rat Na^+/H^+ antiporter activity and NHE3 mRNA and protein abundance. To this end, we examined whether adrenalectomy prevents the maturation of Na^+/H^+ antiporter activity and NHE3 mRNA and protein abundance and whether physiologic glucocorticoid replacement in adrenalectomized animals returns the levels to that of sham-operated controls.

METHODS

Animals

Sprague-Dawley rats underwent either adrenalectomy (ADX) or a sham operation at nine days of age, a time

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well before the postnatal increase in glucocorticoid levels [27]. Bilateral adrenalectomy was performed under light ether anesthesia using two lumbodorsal incisions. After gently excising each adrenal gland, the incision was closed in two layers. The muscle was sutured using 4-0 silk, and the skin layer was closed with stainless steel surgical clips. During the surgery, the instruments used were rinsed with ethanol before each entry into the anesthetized pup to prevent stray adrenal cells from surviving and forming colonies within the abdominal cavity [28]. A similar procedure was conducted for sham surgical procedures, except that the adrenal glands were left undisturbed. The pups were allowed to recover on a warm pad for at least two hours before they were placed with their mother. Soon after recovery, adrenalectomized animals were injected subcutaneously with 100 μg of the long-acting mineralocorticoid deoxycorticosterone pivalate (Percorten-V; Novartis Animal Health Inc., Greensboro, NC, USA) in a 0.9% saline suspension. Supplemental injections of 150 and 200 μg were administered on days 16 and 23, respectively [29]. Sham-operated rats received an equal volume of 0.9% saline injections. The ADX rats were divided into corticosterone replacement (ADX-Cort) and untreated (ADX-vehicle) groups. The ADX-Cort animals received a daily subcutaneous injection of corticosterone (8 μg/gm of body weight) from day 14 onward until the rats were studied at day 30 of age [30, 31]. The ADX-vehicle group was injected with an equivalent volume of vehicle. All sham rats received comparable vehicle injections. Animals were weighed on day 30 of life prior to sacrifice. Complete adrenalectomy was confirmed by measuring plasma corticosterone levels. To maximize the detection of incompletely adrenalectomized animals, all adrenalectomized animals were stressed by a loud clap of the hands five minutes before sacrifice [28]. Serum corticosterone levels were determined by using corticosterone (^{3}H) RIA Kit (ICN Biomedicals Inc., Costa Mesa, CA, USA).

In vitro microperfusion

We have recently demonstrated that 0.2 to 0.5 mm neonatal and young rat proximal convoluted tubules can be dissected free hand without collagenase and perfused in vitro [6]. Isolated segments of neonatal and adult rat proximal convoluted tubules were perfused using concentric glass pipettes using techniques previously described for rabbit proximal tubules [2, 4, 8, 32]. Briefly, rat proximal convoluted tubules were dissected from 9-day-old sham, 30-day-old sham, 30-day-old ADX, and 30-day-old ADX-Cort in Hank's balanced salt solution containing (in mmol/L) 137 NaCl, 5 KCl, 0.8 $MgSO_4$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 1 $MgCl_2$, 10 Tris (hydroxymethyl) aminomethane hydrochloride, 0.25 $CaCl_2$, 2 glutamine, 2 heptanoic acid, and 2 lactate at 4°C (pH 7.4). Tubules were transferred to a 0.2 mL chamber in which the bathing solution was preheated to 38°C.

Measurement of pH_i

The composition of the solutions used in these experiments is shown in Table 1. The fluorescent dye 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) was used to determine the change in cell pH (pH_i) as described previously [2, 6, 8, 32, 33]. pH_i was measured with a Nikon inverted epifluorescent microscope attached to a PTI Ratiomaster at a rate of 30 measurements per second. A variable diaphragm was placed over the area to be measured. Intracellular pH_i was determined from the ratio of fluorescence (F_{500}/F_{450}) using a nigericin calibration curve as previously described [32–34].

Measurement of proton flux rates J_H

Proton flux rates (all proton fluxes are presented as absolute values expressed as J_H in pmol/mm · min) resulting from a luminal fluid change were calculated using the following formula:

$$J_H = \frac{dpH_i}{dt} \cdot \frac{V}{mm} \cdot \beta$$

where dpH_i/dt is the rate of initial change in pH_i after a luminal fluid change, V is the tubular volume in L/mm, and β is the buffer capacity. Tubular volume was calculated from the measured inner and outer tubular diameters at ×400 magnification using an eyepiece reticle. Tubular volumes of proximal convoluted tubules from 9-day-old, 30-day-old ADX, 30-day-old ADX-Cort, and 30-day sham were 591 ± 60, 684 ± 25, 1044 ± 62, and 1107 ± 44 × 10^{-12} L/mm, respectively. The tubular volumes of the 9-day-old and the 30-day-old ADX groups were smaller that the ADX-Cort and 30-day-old sham ($P < 0.05$). Apparent buffer capacity (β) was measured previously using NH_3/NH_4^+ [6]. Solutions (D and E) were used for measurement of apparent buffer capacity. These solutions did not contain Na^+ or Cl^- so as to inhibit all acidification mechanisms due to Na^+ and Cl^--dependent transporters. In the presence of 25 mmol/L HCO_3, buffer capacity was 98.7 ± 16.4 mmol/L/pH in neonatal proximal convoluted tubules (PCT) and 98.9 ± 17.6 mmol/L/pH in adult PCT [6].

Tubules were incubated with an ultrafiltrate-like solution in the lumen and bath (solution B containing 5 mmol/L glucose and 5 mmol/L alanine) for at least five minutes before loading with 5 × 10^{-6} mol/L BCECF, and tubules had a constant pH_i for several minutes prior to the measurement of the transporter activity. dpH_i/dt was measured from the slope of change in pH_i immediately after a luminal fluid change. Steady-state pH_i values were reached within one minute after a luminal fluid exchange, but pH_i was measured for several minutes to ensure that a steady-state pH_i was achieved.

Table 1. Solutions used in intracellular pH studies

		Luminal		No Na	
	Bath solution A	Na^+ solution B	No Na^+ solution C	No Cl^- no NH_4^+ D	No Cl +20 mmol/L NH_4^+ E
NaCl	140	115	–	–	–
$NaHCO_3$	5	25	–	–	–
TMA-OH	–	–	–	115	95
Gluconic acid Lactone	–	–	–	115	115
NMDG-Cl	–	–	115	–	–
Choline HCO_3	–	–	25	25	25
NH_4OH	–	–	–	–	20
KCl	5	–	–	–	–
K_2HPO_4	–	2.5	2.5	2.5	2.5
$MgCl_2$	–	1	1	–	–
$MgSO_4$	1	–	–	–	–
Na_2HPO_4	1	–	–	–	–
Glucose	5	–	–	5	5
L-Alanine	5	–	–	5	5
Urea	5	–	–	–	–
Mg gluconate	–	–	–	1	1
$CaCl_2$	1.8	1.8	1.8	–	–
Ca gluconate	–	–	–	12.5	12.5
Heptanoic acid	2	–	–	–	–
pH	6.6	7.4	7.4	7.4	7.4

All constituents are in mmol/L. All solutions were adjusted to an osmolality of 295 mOsm/kg H_2O and were bubbled with 5% CO_2-95% O_2. Abbreviations are: pH_i, intracellular pH; TMA, tetramethyl ammonium; NMDG, N-methyl-D-glucamine.

The Na^+/H^+ antiporter activity was measured as previously described in adult rat proximal convoluted tubules perfused in vivo and neonatal and adult rabbit and rat proximal convoluted tubules perfused in vitro [4, 6, 8, 32, 33, 35]. Neonatal and adult rat tubules were perfused with an ultrafiltrate-like solution without glucose and amino acids (solution B). Organic solutes were omitted from the luminal solution because sodium coupled glucose and amino acid transport depolarizes the basolateral membrane, which may affect bicarbonate exit, an electrogenic process [33]. 4-Acetamido-4′-isothiocyanostilbene-2,2′ disulfonic acid (1 mmol/L SITS) was present in the bathing solution to inhibit the sodium bicarbonate cotransporter, a major regulator of intracellular pH_i in proximal convoluted tubules [32, 33]. The bathing solution had a bicarbonate concentration of 5 mmol/L and a pH of 6.6 to compensate for the cell alkalinization caused by the addition of bath SITS [32, 33, 35]. The bathing solution was exchanged at a rate of at least 5 mL/min. Under these conditions, changes in pH_i in response to a change in luminal sodium concentration are a measure of Na^+/H^+ antiporter activity [6, 8, 32, 35]. In the experimental period, luminal sodium was removed (solution C).

Brush-border membrane vesicle isolation

Kidneys were removed and placed in an ice-cold isolation buffer containing 300 mmol/L mannitol, 16 mmol/L N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 5 mmol/L ethylene glycol-bis (β-aminoethyl ether) N,N,N′N′-tetraacetic acid (EGTA) titrated to pH 7.4 with Tris. The isolation buffer contained aprotinin (2 μg/mL), leupeptin (2 μg/mL), and phenyl-methylsulfonyl fluoride (100 μg/mL). The cortex was homogenized with 20 strokes of a Potter Eljevhem homogenizer at 4°C. Brush-border membrane vesicles (BBMVs) were then isolated by differential centrifugation and magnesium precipitation as described previously [6]. The final BBMV fraction was resuspended in isolation buffer. Protein was assayed using the Lowry method with crystalline BSA as the standard [36].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting

Brush-border membrane proteins (30 μg/lane) were denatured and then separated on a 7.5% polyacrylamide gel using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described [6]. The proteins were transferred overnight to a polyvinylidiene difluoride membrane at 120 to 140 mA at 4°C. The blot was blocked with fresh Blotto (5% nonfat milk and 0.1% Tween 20 in PBS, pH 7.4) for one hour followed by incubation with primary antibody to NHE3. NHE3 antibody, a generous gift from Orson Moe, M.D, was a rabbit polyclonal antibody directed against a fusion protein of maltose-binding protein and rat NHE3 amino acids 405 to 831 [22]. NHE3 antibody was added at 1:250 dilution for one hour at room temperature. The blot was

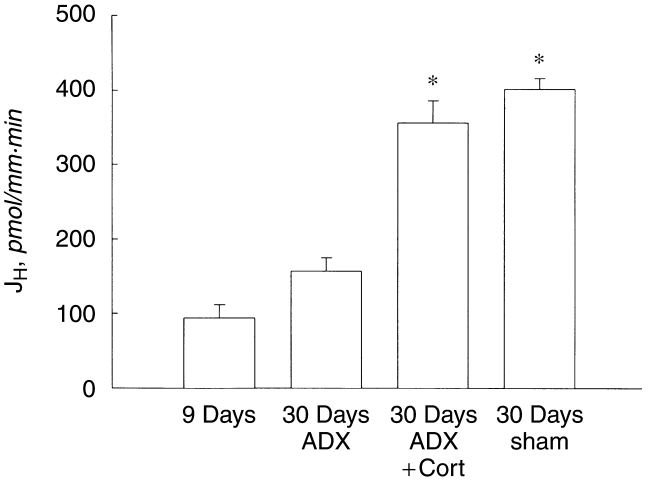

Fig. 1. Na^+/H^+ antiporter activity in rat proximal convoluted tubules perfused in vitro. J_H was measured after removal of luminal sodium. Na^+/H^+ antiporter activity in the 9-day-old group and the 30-day-old ADX group was less than that of the 30-day-old ADX-Cort and the 30-day-old sham. *$P < 0.05$ vs. 9-day and 30-day ADX.

then washed extensively with Blotto. The secondary antibody, horseradish peroxidase-conjugated donkey anti-rabbit immunoglobulin, was added at 1:10,000 dilution and incubated in room temperature for one hour. The blot was again washed with Blotto, and enhanced chemiluminescence was used to detect bound antibody (Amersham Life Science, Arlington Heights, IL, USA). The NHE3 protein abundance was quantitated using densitometry. Equal loading of the samples was confirmed using an antibody to β-actin at a 1:5000 dilution (Sigma Biochemicals and Reagents, St. Louis, MO, USA).

RNA isolation and analysis

Slices of renal cortex from decapsulated kidneys were homogenized in RNAzol [1:1, phenol-RNAzol stock (4 mol/L guanidine thiocyanate, 25 mmol/L disodium-citrate, pH = 7.0), 0.5% sarcosyl] containing 3.6 μL/mL β-mercaptoethanol. RNA was extracted in presence of 3 mol/L NaOAc (pH 4.0) and chloroform, purified using isopropanol precipitation, and washed twice with 80% ethanol [37]. Oligo (dT) column chromatography was used to purify poly $(A)^+$ RNA from total RNA. Poly $(A)^+$ RNA was quantitated with an LKB Ultra-spec III spectrophotometer at 260 nm, and 5 μg were fractionated using agarose-formaldehyde gel electrophoresis and were then transferred to a nylon filter (GeneScreen Plus; New England Nuclear, Boston, MA, USA). The filter was prehybridized at 42°C for four hours with 5 × standard saline citrate (SSC), 5 × Denhardt's solution (Ficoll, bovine serum albumin and polyvinylpyrrolidone, each at 1 mg/mL), 0.5% SDS, and 0.5 mg/mL of sheared salmon sperm DNA and then hybridized to double-stranded uniformly ^{32}P-labeled cDNA probes ($>10^6$ cpm/mL) in this hybridization solution at 42°C for approximately 16 hours. The probes were synthesized by random hexamer method using 50 to 100 ng of cDNA: NHE3 was the rat 1.2 kb *Pst* I fragment [12], and GAPDH was a 1.2 kb *Hin*dIII/BamHI fragment. The filter was then washed with 2 × SSC twice and 0.1% SDS for five minutes at room temperature and then with 0.1 × SSC and 1% SDS at 55°C for 40 minutes two additional times. NHE3 and GAPDH mRNA abundance was quantitated by autoradiography and densitometry.

Table 2. Effect of luminal Na^+ removal on pH_i in rat PCT

	N	Na^+	ONa^+	Na^+
9-day PCT	12	7.49 ± 0.05	7.18 ± 0.07[a]	7.42 ± 0.07
30-day ADX PCT	8	7.67 ± 0.11	7.13 ± 0.14[a]	7.67 ± 0.11
30-day ADX + steroid	7	7.53 ± 0.08	6.97 ± 0.08[a]	7.36 ± 0.09
30-day sham	6	7.59 ± 0.03	6.83 ± 0.13[a]	7.59 ± 0.04

Data are pH_i. Abbreviations are: PCT, proximal convoluted tubule; pH_i, change in cell pH; ADX, adrenalectomy.
[a] $P < 0.01$ compared to sodium-containing solutions

Statistical analysis

Each experiment was performed at least four times. Data are expressed as mean ± SEM. Statistical significance was determined using analysis of variance (ANOVA).

RESULTS

Efficacy of adrenalectomy

Serum corticosterone concentrations at the time of sacrifice in the nine-day-old, ADX, ADX ± Cort, and sham-operated groups were 0.34 ± 0.07, 0.28 ± 0.09, 1.02 ± 0.39, and 24.14 ± 4.21 μg/dL, respectively. The ADX ± Cort group received their last corticosterone injection 14 hours prior to sacrifice. The sham group had a higher corticosterone level than the other groups ($P < 0.001$). These data show that the maturational increase in serum glucocorticoid levels was prevented by adrenalectomy.

The weight of 9-day-old, 30-day ADX, 30-day ADX ± Cort, and sham-operated rats was 24.8 ± 0.3, 63.8 ± 1.3, 87.9 ± 1.8, and 91.9 ± 1.6 g, respectively. Consistent with previous studies, adrenalectomized rats had a significantly lower body weight than their sham counterparts and the ADX ± Cort group ($P < 0.001$) [28, 29]. Corticosterone replacement restored body weight in ADX group to levels comparable to sham.

Effect of glucocorticoids on Na^+/H^+ exchanger in PCT

The rate of proximal tubule proton secretion caused by luminal sodium removal in the four groups is shown in Figure 1, and the steady state pH_i is shown in Table 2. As can be seen, the proton secretory rate was significantly slower in the 9-day-old and 30-day-old ADX groups than

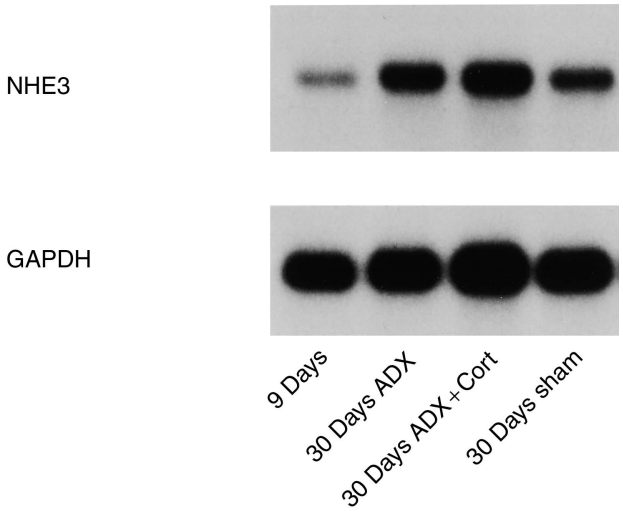

Fig. 2. Effect of glucocorticoids on NHE3 mRNA abundance. Northern blot with 5 μg of poly(A) RNA from renal cortex of rats of ages shown previously in this article. GAPDH mRNA is shown for comparison. NHE3 mRNA abundance was significantly less in the nine-day-old group than that of the other groups ($P < 0.01$).

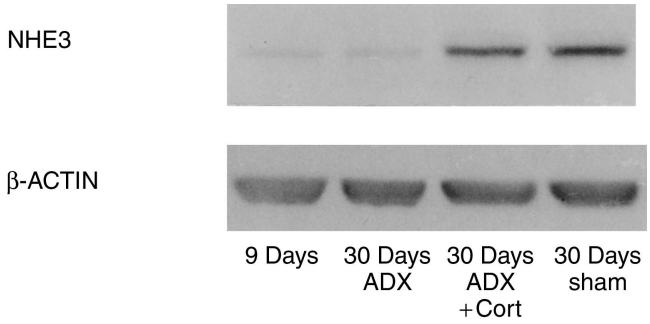

Fig. 3. Effect of glucocorticoids on Na^+/H^+ antiporter (NHE3) protein abundance from renal brush-border membrane vessicles (BBMVs) compared with β-actin. NHE3 protein abundance was comparable in the 9-day-old and ADX group and less than that in the 30-day-old ADX-Cort and the 30-day-old sham ($P < 0.001$).

in the 30-day-old ADX ± Cort and sham-operated rats. Using ANOVA and comparing all four groups together, there was no difference between the nine-day-old group and the ADX group. However, if these two groups alone were compared, the rate of proton secretion with luminal sodium removal was higher in the ADX group that the nine-day-old group, indicating that ADX did not totally prevent maturation of the Na^+/H^+ exchanger.

Effect of glucocorticoids on renal cortical NHE3 mRNA and protein abundance

To delineate the role of circulating glucocorticoids on renal cortical NHE3 mRNA abundance, 9-day-old, 30-day-old ADX, 30-day-old ADX ± Cort, and 30-day-old sham control groups were compared using Northern blot analysis. The results are shown in Figure 2. The ratio of NHE3 mRNA to GAPDH abundance was 0.85 ± 0.24 in the nine-day-old group, which was less than the ADX (3.78 ± 0.79), ADX ± Cort (4.55 ± 0.57), and sham-operated (3.90 ± 0.74) groups ($P < 0.01$). There was no difference in the ratio of NHE3 to GAPDH mRNA abundance in ADX, ADX ± Cort, and sham-operated control groups. Thus, adrenalectomy did not prevent the maturational increase in renal cortical NHE3 mRNA abundance.

Finally, we examined the NHE3 protein abundance in BBMV using Western blot analysis. The results are shown in Figure 3. The ratio of NHE3 protein to β-actin in the 9-day-old group was 0.10 ± 0.03, which was not different than that of 30-day-old ADX group (0.12 ± 0.05). However, these two groups had significantly lower values than that of 30-day-old ADX ± Cort group (0.63 ± 0.04) and 30-day-old sham-operated group (0.60 ± 0.02, $P < 0.001$). Thus, ADX in neonatal animals prevented the maturational increase in BBMV NHE3 abundance.

DISCUSSION

The present study examined whether ADX in rats prior to the postnatal increase in serum glucocorticoids would prevent the maturational increase in Na^+/H^+ antiporter activity and NHE3 mRNA and protein abundance. We found that ADX rats at 30 days of age had a comparable brush-border membrane NHE3 protein abundance but a somewhat higher Na^+/H^+ antiporter activity as compared with that of 9-day-old neonates. The rate of Na^+/H^+ antiporter activity in both these groups was much lower than that in either the ADX-Cort or the sham-operated control group. NHE3 mRNA abundance was lower in the nine-day-old rats than the control, but adrenalectomy did not prevent the maturational increase in NHE3 mRNA abundance.

The factors that cause the postnatal increase in proximal tubule Na^+/H^+ antiporter activity are not known. We have previously examined the role of thyroid hormone in the maturation of NHE3 and Na^+/H^+ antiporter activity [38]. In this study, neonatal rats were made hypothyroid by administering water containing 0.01% propylthiouracil from 14 days' gestation until the day of study at 21 days of age. Hypothyroid rats were compared with euthyroid controls and hypothyroid animals given pharmacologic thyroid replacement for four days. While there was a reduction in BBMV Na^+/H^+ antiporter activity and NHE3 protein abundance in hypothyroid animals and an increase in hyperthyroid animals compared with euthyroid controls, the effect of hypothyroidism and hyperthyroidism on Na^+/H^+ antiporter activity was trivial. In addition, while hyperthyroid rats had an increase in NHE3 mRNA abundance greater than control, hypothyroid neonates had no reduction in NHE3 mRNA abundance. These data

were consistent with a small role for thyroid hormone in the postnatal maturation of the Na^+/H^+ antiporter.

Glucocorticoids have been shown to affect proximal tubule acidification. The administration of dexamethasone in adult rats and adrenalectomized adult rats has been shown to stimulate the rate of Na^+/H^+ antiporter activity in renal BBMV without altering the affinity of the antiporter for Na^+ or H^+ [39–41]. However, adrenalectomized animals did not have a reduced rate of Na^+/H^+ antiporter activity [41]. Consistent with these studies, the administration of aminoglutethimide to adult rabbits produced glucocorticoid deficiency that did not affect renal cortical NHE3 mRNA abundance [42].

In addition to potential hemodynamic effects, glucocorticoids have a direct effect on the proximal tubule to stimulate acidification [43]. The addition of dexamethasone to the bathing solution of proximal convoluted tubules perfused in vitro resulted in an increase in the rate of bicarbonate transport after three hours of incubation [43]. The stimulation in bicarbonate transport by dexamethasone was not present when proximal tubules were incubated with either actinomycin D or cycloheximide [43]. A direct epithelial effect on Na^+/H^+ antiporter activity was demonstrated in rabbit proximal tubules incubated with dexamethasone in vitro and in OKP cells [44, 45]. The promoter for NHE3 gene has glucocorticoid response elements, and dexamethasone has been shown to increase Na^+/H^+ antiporter activity in OKP cells by increasing the rate of transcription [45, 46].

In the present study, we find that adrenalectomized rats had comparable amount of NHE3 mRNA to sham-operated controls, but significantly less NHE3 protein in BBMVs. The discordance between brush-border membrane NHE3 and NHE3 mRNA abundance suggests that glucocorticoids may have an effect on NHE3 processing besides regulating NHE3 transcription. Glucocorticoids have been shown to affect mRNA stability [47, 48]. However, we have previously examined whether glucocorticoids affect NHE3 mRNA stability in OKP cells and found that the glucocorticoid-mediated increase in mRNA abundance was entirely due to an increase in transcription [45].

Glucocorticoids have been shown to affect translation and post-translational processing of a number of proteins. Dexamethasone increases translation efficiency of glutamine synthase, myelin basic protein, and the α1- and α2-, but not the β1 subunits of the Na,K-ATPase [49–51]. Glucocorticoids also increase secretion of atrial natriuretic peptide from atrial myocytes, an effect blocked by RU486, a glucocorticoid receptor antagonist [52]. Murine mammary tumor virus envelope glycoproteins have been used as a probe to investigate post-transcriptional regulation by glucocorticoids [53–57]. Glucocorticoids regulate viral protein processing in infected rat hepatoma cells by modulating glycoprotein processing and phosphoprotein maturation as well as trafficking of viral glycoproteins to the cell surface [53–56]. The glucocorticoid effect on trafficking of viral glycoproteins is dependent on the glucocorticoid receptor [54]. In addition, glucocorticoids have been show to increase the half-life of some glycosylated murine mammary tumor viral proteins [57].

There is evidence that glucocorticoids play a role in post-transcriptional regulation of NHE3. NHE3 is present in apical membranes as well as subapical endosomal compartments of proximal tubule cells [58–60]. In OKP cells, NHE3 is expressed on the apical membrane and NHE3 mRNA and protein abundance and Na^+/H^+ antiporter activity are all increased by glucocorticoids and by incubation in acid media [44, 45, 61]. Glucocorticoids and acid media have a synergistic effect to increase NHE3 protein abundance independent of their effect to increase NHE3 transcription [61]. This synergistic effect of glucocorticoids and acid media is in part due to an increase in trafficking of NHE3 to the apical membrane. Future studies are needed to elucidate whether the disparity in brush-border membrane NHE3 protein abundance and mRNA in adrenalectomized rats and control animals is due to a glucocorticoid-dependent step in NHE3 trafficking.

Previous studies have examined whether glucocorticoids play a role in the maturation of proximal tubule acidification. In these studies, the administration of pharmacologic doses of dexamethasone to pregnant rabbits late in gestation resulted in an increase in the rate of fetal renal BBMV Na^+/H^+ antiporter activity [62]. The administration of prenatal dexamethasone increases rabbit neonatal proximal convoluted tubule bicarbonate absorption, Na^+/H^+ antiporter activity, and renal cortical NHE3 mRNA and protein abundance [4, 26]. The administration of dexamethasone to neonatal rabbits has also been shown to increase renal cortical NHE3 mRNA and protein abundance [26]. While these studies are consistent with a role for glucocorticoids in the maturation of proximal tubule acidification, they suffer from a number of problems addressed in the current study. These studies used pharmacologic doses of dexamethasone, a steroid that is a poor substrate for 11β-hydroxysteroid dehydrogenase (11β-HSD) and that is present in the proximal tubule. Finally, these studies do not address whether prevention of the maturational increase in glucocorticoids prevents the increase in Na^+/H^+ activity or NHE3 mRNA or protein abundance.

11β-Hydroxysteroid dehydrogenase inactivates corticosterone and cortisol by converting these glucocorticoids to 11 dehydrocorticosterone and cortisone, respectively [63–66]. Two isoforms of 11β-HSD have been identified. 11β-HSD-2 is colocalized with mineralocorticoid receptors in the distal nephron and prevents activation of mineralocorticoid receptors by glucocorticoids that are potential agonists for the receptor [63–66]. 11β-HSD-1

is present in the proximal tubule. However, the activity of 11β-HSD-1 is far less in neonatal than in adult proximal tubules, which is likely a factor that permits activation of circulating glucocorticoids to act on this segment during nephron maturation [67]. There is also evidence that 11β-HSD-1 can function as a reductase in vivo, regenerating active glucocorticoids in intact cells [68–70].

Our study used doses of corticosterone that have previously been shown to provide physiologic glucocorticoid replacement in adrenalectomized rats [30, 31]. Measurements of corticosterone levels 14 hours after the last dose were lower than that of sham-operated animals, while the weight of the ADX animals with corticosterone replacement was greater than that of ADX animals and was comparable to sham-treated animals. Both Na^+/H^+ antiporter activity as well as NHE3 protein abundance were comparable in the 30-day-old ADX-Cort group and sham-operated control group, in contrast to 30-day-old ADX group, which had levels comparable to that of the 9-day-old group. Thus, exogenous corticosterone replacement restored proximal tubule Na^+/H^+ antiporter activity and NHE3 protein abundance in adrenalectomized animals to that of the control group. The adrenalectomized animals were smaller than the sham controls and adrenalectomized rats that received corticosterone replacement, which could have affected other hormones. Thus, glucocorticoid deficiency could have had an indirect effect on NHE3 due to differences in protein caloric intake.

The amount of NHE3 protein reaching the brush-border membrane is affected by the rate of transcription and translation, as well as post-translational processing resulting in insertion of the protein into the apical membrane. We found that, although NHE3 protein abundance is reduced in ADX rats, there was no corresponding decrease in NHE3 mRNA abundance. In previous studies of adrenalectomized rats, there was an increase in plasma thyrotropin-releasing hormone (TRH), TSH, T_4, T_3 levels, which may compensate for the reduced glucocorticoid levels in adrenalectomized rats [71]. Consistent with this is our recent finding that thyroid hormone-like glucocorticoids increase NHE3 transcription [72].

In these studies, the weight of the adrenalectomized rats was 30% less than that of the controls. It is possible that a difference in caloric or protein intake could be a factor to explain our results. In addition, it is also possible that glucocorticoid deficiency may affect other hormones that regulate NHE3. However, we have previously shown that hypothyroid rats, which had a 47% reduction in weight, had only a trivial reduction in Na^+/H^+ antiporter activity compared with euthyroid controls [38]. Thus, protein calorie deficiency is unlikely to be a major factor to explain our results.

In summary, our data show that prevention of the normal postnatal maturational increase in glucocorticoids significantly attenuates the maturational increase in NHE3 activity and prevents the increase in BBMV protein abundance. Adrenalectomy did not reduce renal cortical mRNA abundance or totally prevent the maturational increase in Na^+/H^+ antiporter activity. These data demonstrate that glucocorticoids are important in the postnatal maturation of the Na^+/H^+ antiporter. However, these data show that other factors are involved in the postnatal maturation of renal acidification or have a compensatory role in the face of glucocorticoid deficiency.

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Reprint requests to Michel Baum, M.D., Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9063, USA.
E-mail: mbaum@mednet.swmed.edu

APPENDIX

Abbreviations used in this article are: ADX, adrenalectomy; BBMV, brush-border membrane vesicles; BCECF, 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein; 11β-HSD, 11β-hydroxysteroid dehydrogenase; BSA, bovine serum albumin; EGTA, ethylene glycol-bis (β-aminoethyl ether) N,N,N′,N′-tetraacetic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; J_H, proton flux rate; Cort, corticosterone; NHE, Na^+/H^+ exchanger; PBS, phosphate-buffered saline; PCT, proximal convoluted tubule; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SITS, 4-acetamido-4′-isothiocyano-stilbene-2,2′ disulfonic acid; SSC, standard saline citrate.

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